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Indirect Spectrophotometric Method for Estimation of Irbesartan in Pure and in Pharmaceutical Dosage Form Using Oxidation and Reduction Reaction

Zahraa Mohammed Thamir, Farha Khalaf Omar

Department of Chemistry, College of Education for Girls, University of Mosul, Iraq.

A R T I C L E   I N F O

A B S T R A C T

Spectrophotometric method was developed for estimation of Irbesartan (IRB) in pure form and in pharmaceutical preparations. The idea of our procedure is oxidation of IRB with recognised excess amount of N-bromosuccinimide (NBS) in acidic medium and formerly remaining NBS is estimated by Rhodamine (RD) dye and then we measure the absorbance of remaining dye at 577 nm. A calibration curve was linear over the concentration range 1 –30 μg.ml-1 of IRB with correlation coefficient equal to 0.9991. The molar absorptivity were determined to be 4.499 × 104 L.mol-1.cm-1 and sandell's index value 0.0095 μg.cm-2. We are calculated the(LOD) = 0.469 μg.ml-1and (LOQ) = 1.982,.The our proposed method raped, sensitive and very selective and has been applied to the estimation of IRB in obtainable dosage form. The recoverability and selectivity of the proposed method were confirmed by applying a standard addition procedure.

1. Introduction

Irbesartan, (IRB) chemical name is 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl] phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one, (IRB) is a white to off-white crystalline powder with a molecular weight of 428.5 g/mol. It is a nonpolar compound therfore IRB is slightly soluble in alcohol and methylene chloride and essentially insoluble in water (Laxmi *et al.*, 2013).
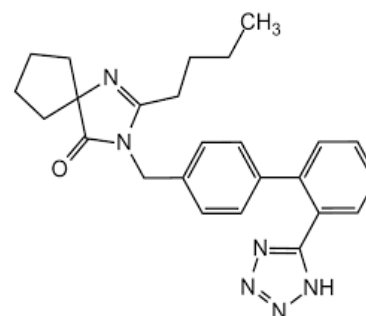
Scheme. 1: chemical structure of Irbesartan $(C_{25}H_{28}N_6O)$ M.Wt. = 428.53 g/mol

Corresponding author:
E-mail addresses: zahraa.20gep73@student.uomosul.edu.iq (Zahraa), fkzz4@uomosul.edu.iq (Farha)

IRB is a protected receptor agonist and is a drug used to treat high blood pressure. In fact, IRB reduces heart rate at daily dose and biotransformation system in humans and has shown a good response in the treatment of diabetic nephropathy.(Theodore *et al.*, 1998). IRB can mainly be used to treat high altitude congestive heart failure and chronic renal failure.(Weber , 1997),

Previous literature was searched and many methods were explored to determine IRB either in pure form or in pharmaceutical preparations, directly or indirectly for IRB, including: Spectrophotometric methods (Syeda *et al* ,2011) , (Khalid *et al.* , 2017) , (Jadhav *et al.* , 2014) and (Biyani and Yadav, 2019), LC-MS (Qui *et al.* , 2014) and(Chi and Chia 2011), HPLC (Vujić *et al.* , 2012), (Alanazi *et al.* , 2014) , (Koyuturk *et al.* , 2014) and(Goswami , 2014).

A rational, fast and easy spectrophotometric method for the determination of IRB in pharmaceutical preparations, which is routinely used for quality control checks of drugs, with no adverse effect of other additives present in these preparations, has been developed and improved, and the method has been successfully applied for the determination of IRB in tablets.

2. Experimental

Apparatus

All absorption spectra and absorbance measurements were done by using a double beam UV-visible spectrophotometer (JASCOV-630) with 1.0-cm quartz cells.

Chemical reagents

All of the chemical compounds utilized in the tests were analytical grade, which meant they didn't need to be purified any further.

IRB stock solution 1000 μg. ml⁻¹: Prepared by dissolving 0.1 g of pure IRB in μml of ethanol solution with slightly heating and shaking for 5 min, then diluted to 100 ml with distilled water using a volumetric flask. Working **standard solution (100 μg. ml⁻¹) (3.386×10^{-4} mol.L⁻¹)** was produced by diluting the stock solution appropriately.

N-bromosuccinamid solution 100 μg. ml⁻¹: It was made by combining 0.01g of NBS with distilled water and diluting it to 100 ml with distilled water. For at least two days, this solution remained stable.

Rhodamine (RD) stock solution 1000 μg. ml⁻¹ : It was made by dissolving 0.1 g of dye powder in distilled water and diluting to 100 ml with the same solvent**. Working standard solution (100 μg. ml⁻¹) (2.087×10^{-4} mol.L⁻¹)** was prepared by diluting the stock solution with distilled water to the desired concentration.

Surfactants solutions (1% m/v): Prepared by dissolving 1.00 g in 100 ml of distilled water by stirring and heating.

hydrochloric acid 1 mol.L⁻¹: It was prepared by diluting 8.47 ml of concentrated hydrochloric acid to 100 ml with distilled water.

Initial procedure

In 25 ml volumetric flask we added 1.0 ml of 100 μg. ml⁻¹ of IRB , followed by 1.0 ml of 100 g. ml⁻¹ NBS solution and after standing for few minutes we added 1.0 ml of 100 μg. ml⁻¹ rhodamine dye and then added 1.0 ml 1 mol.L⁻¹ HCl,. We noticed that a red color gradually appeared over a short period, with the highest absorption peak at 577 nm and stable at room temperature.

Procedure for dosage forms

Recommended procedure to assay IRBN in drug

IRB tablets (GIZAN) solution 100 μg/ml : Five tablets (300 mg IRB/tablet) were weighed and crushed into a fine powder, then a portion 0.017g from the powder equivalent to 0.01 g of pure IRBN was weighed and dissolved in 5 ml ethanol by heating and will be stirred, then filtered with filter paper, the filter transferred to a 100 ml volumetric flask then the volume is completed to the mark using water distilled; This solution was treated as a generic procedure.

3. Results and Discussion

The principle and suggested chemical reaction

From follow – up to the researches of kinetic and mechanism reactions, we find that NBS is an oxidizing agent and a bromination agent in the acid medium for aliphatic and aromatic organic compounds (https: org/chemicals) So suppose a chemical reaction between IRB and calculated amount of NBS:

$$\text{IRB} + \underset{\text{(excess)}}{\text{NBS}} \xrightarrow{\text{H}^+} \underset{\text{(oxidized)}}{\text{IRB}} + \underset{\text{(unreacted)}}{\text{NBS}}$$

Scheme 2

In the second step unreacted NBS (a known quantity) reacts with the RD dye and oxidizes the corresponding amount of the RD dye to a colorless result. Finally, the residual amount of dye proportional to the IRB concentration at 577 nm was measured:

$$\underset{\text{(unreacted)}}{\text{NBS}} + \underset{\text{(excess)}}{\text{RD}} \xrightarrow{\text{H}^+} \underset{\text{(oxidized)}}{\text{RD}} + \underset{\text{(unreacted)}}{\text{RD}}$$

Scheme 3

Optimum Reaction Conditions

All experiments were conducted in 25 ml volumetric flasks with 100 μg of IRB and measuring absorption for colored product at 577nm .

Choose of oxidant agent:

In this study we added 1 ml of available RD-discoloring agents (potassium dichromate,N-bromosuccinamid and potassium iodate) at a concentration (100 μg. ml-1) of each to a number of 25 ml volumetric flasks containing 1.0 ml of 2.087×10^{-4} mol / L^{-1} RD dye and 1.0 ml of 1 M hydrochloric acid, then volume was completed to the mark by distilled water. After bleaching for a few minutes, the absorbance was measured at 577 nm. (Table 1) illustrate that NBS gives the best results, and was therefore selected for subsequent experiments.

Table 1:choose the type of oxidizing agent

Oxidizing agent 100 μg. ml^{-1}	NBS	KIO$_4$	K$_2$Cr$_2$O$_7$
Absorbance	0.086	0.154	0.118

Amount of RD dye

To determine the IRB we must find the largest amount of RD dye that follows Bear's Law. Increasing volumes of 2.087×10^{-4} mol/ L^{-1} RD dye solution were added to a series of 25 ml volumetric flasks containing 1.0 ml (1M) hydrochloric acid. Then complete the volume with distilled water to the mark and measure the absorbance at 577 nm. The standard curve as in (Fig. 1) shows that 2.5 ml of dye is the best volume giving high absorbance within a linear relationship.
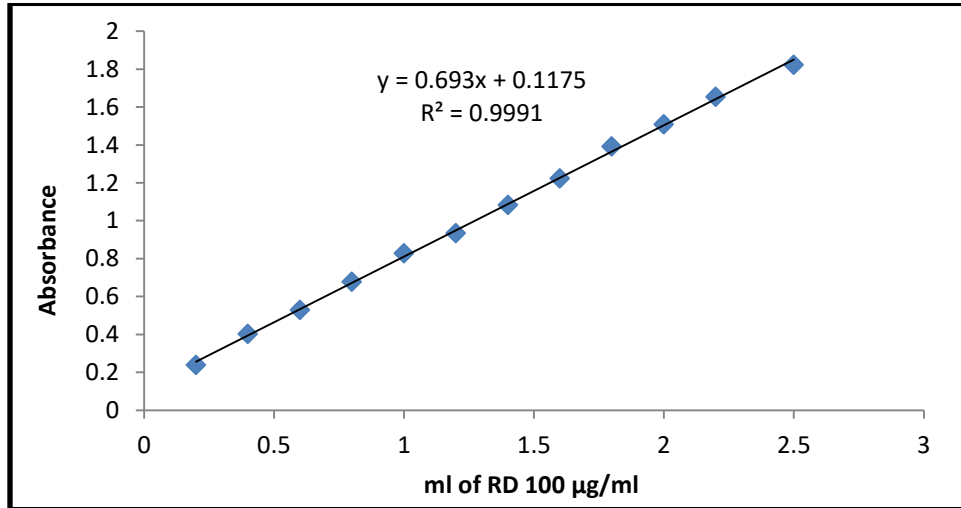
Fig.1: Standard curve for RD dye

Sufficient amount of NBS agent :

This study was done by changing the volume of the NBS reagent while the other parameters were fixed, as it was that 2.0 ml of 100 μg. ml^{-1} NBS is sufficient for decolonization of RD dye   (fig. 2).
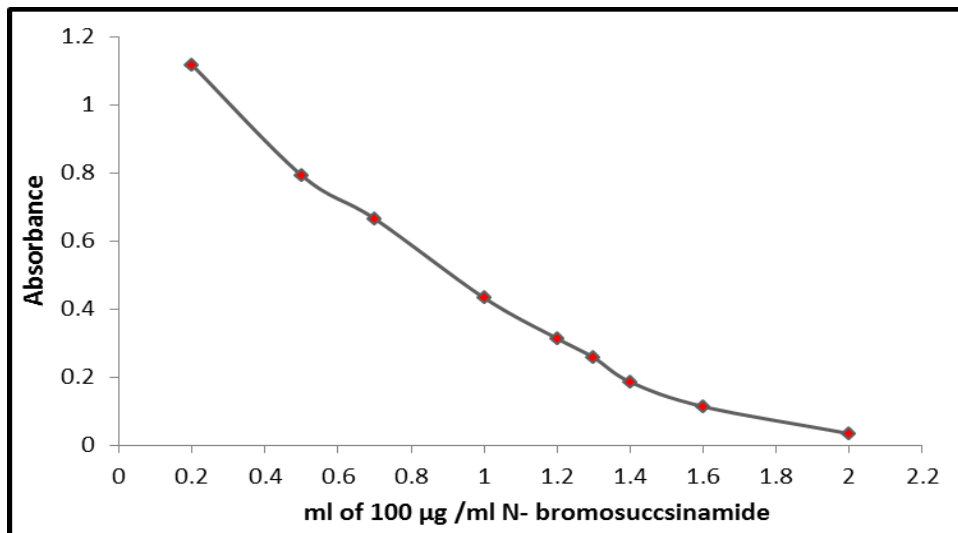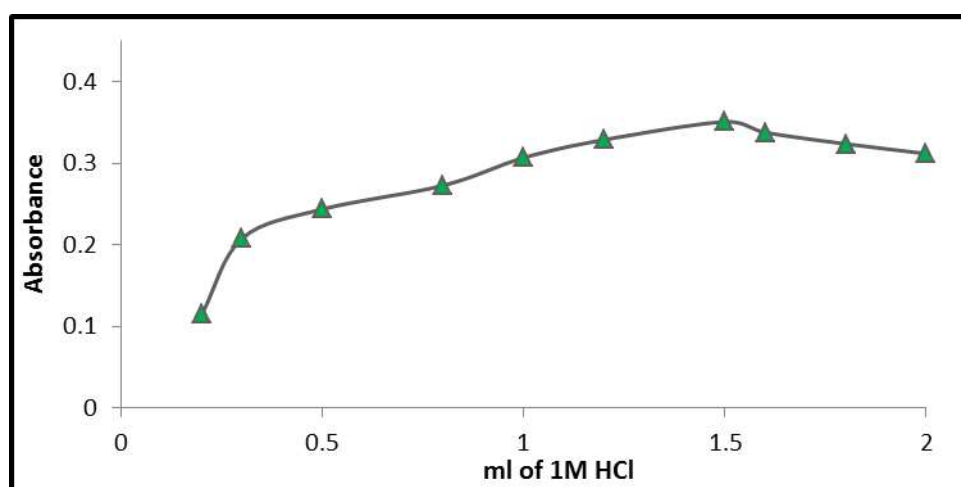

Fig.2: Amount of oxidant agent

Effect of acidic medium:

The number of strong and weak acids was studied to find an ideal acid as a reaction medium. In addition the optimal amount of acid was studied and 1.5 mL of 1 M HCl was chosen as the optimal amount, (Table 2) (Fig. 3)**:**

Table 2:choose the type of acid

Acid solution 1 ml of (1M)	Absorbance
HCl	**0.323**
H_2SO_4	0.276
HNO_3	0.256
CH_3COOH	0.187

Fig. 3 :amount of acid

Effect of time:

The study of the oxidation time is very important, as it was determined by adding the contents of the reaction to a number of 25 ml volumetric flasks, shaking and waiting for different times before adding the RD dye, then waiting for a number of minutes to complete the oxidation reaction before completing the volume to the mark by distilled water.

Table 3: Effect of oxidation time

Standing time before add RD , min	Absorbance / Standing time after add RD and before diluting, min							
	5	10	15	20	30	40	50	60
After addition	0.334	0.346	0.355	0. 359	0.368	0.372	0.370	0.373
5	**0.403**	0. 397	0. 395	0.391	0.393	0.394	0.391	0.396
10	0.389	0.388	0.387	0.385	0.386	0.389	0.394	0.397
15	0.385	0.386	0.385	0.384	0.383	0.389	0.393	0.395

The results in (Table 3) shows that 5 minutes is the best time to complete the oxidation process before adding the RD dye, and that minutes is the waiting time after adding the dye to get the best results.

Effect of surfactants :

This study was carried out using several different types of surfactants, with a concentration of 1%

m/v for each of them as well as different amounts of each type. (table 5).

Several types of surfactants have been studied, Where it was found that these materials do not have a positive effect on the absorption of the colored product, but we found that they reduce the absorption as shown in (Table 4), so these materials were excluded in subsequent experiments.

Table 4: Effect of surfactants

Surfactant 1% m/v	Absorbance / ml of Surfactant				
	0.0	0.5	1	1.5	2
Triton X 100	0.402	0.388	0.375	0.367	0.330
SDS		0.368	0.339	0.322	0.314
CTAB		0.381	0.377	0.359	0.336
CPC		0.340	0.341	0.324	0.310

Effect of temperature and stability :

The effect of temperature on the stability of the colored product was studied by measuring the absorption at different temperatures, and it was

found that the absorption of the colored product was stable for at least an hour at room temperature (25°C ± 2) while the absorption values were unstable at other temperatures such as 5 C^o,50 C^o and 75 C^o , as Shown in Fig. 4:
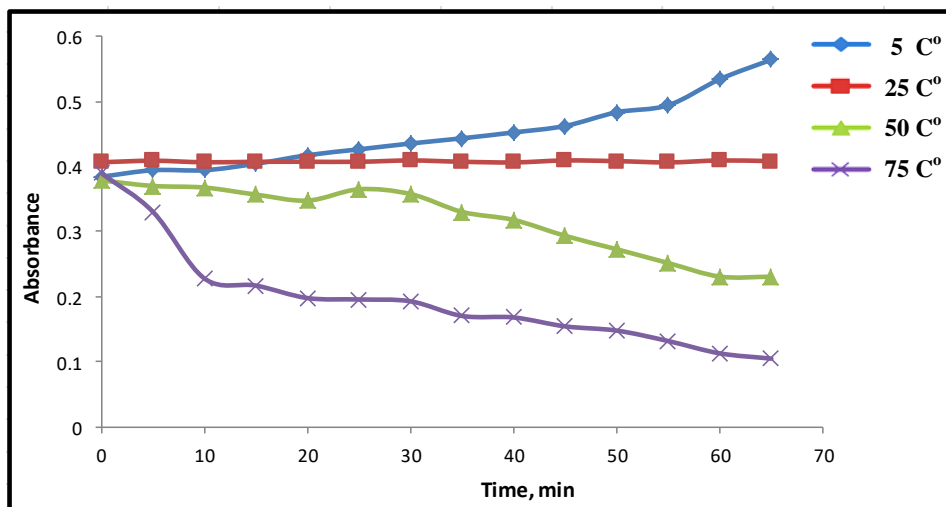
Fig. 4: Effect of heat and time on absorbance of colored product

Sequence of additions :

It was studied whether the order of addition had an effect on the absorption value of the stained product and the results are shown in Table 5.

Table 5: Effect of Sequence of additions

N.O	Sequence of additions	Absorbance
1	**IRB + H+ + OX + RD**	**0.415**
2	IRB + OX + H+ + RD	0.396
3	OX + IRB + H+ + RD	0.264
4	H++ OX + IRB + RD	0.321

From the results presented in Table 5 the best sequence of addition was drug + acid oxidant + red dye. Under the same conditions, the absorbance of ordering other sequences was low.

Final absorption spectrum

Under the optimum conditions and in 25 ml volumetric flask 1 ml of 100 μg. ml^{-1} IRB solution oxidized by excess (2 ml) of 100 μg. ml^{-1} NBS in acidic medium and after standing for 5 min 2.5 ml of 2.087×10^{-4} mol.L^{-1} RD solution was added, then stand at room temperature for 5 min before complete the volume to mark with distilled water. The absorbance was measured for this solution against its blank at 577 nm. (fig. 5) show the spectrum of the colored product of this procedure.
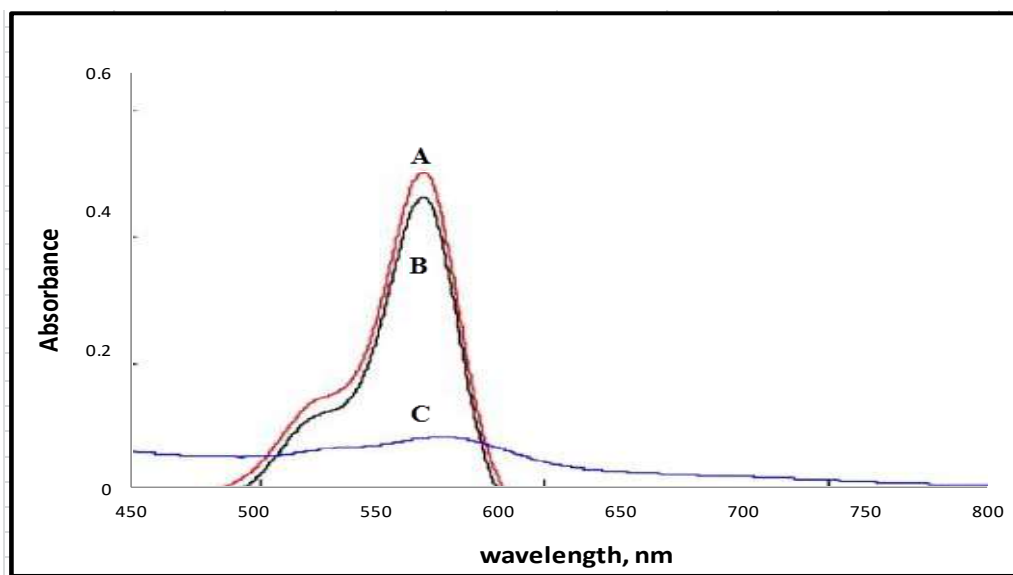

Fig.5: Absorbtion spectrum of 100 μg/25 ml   IRB VS (A) D.W , (B) blank, (C) blanck VS D.W.

The final procedure and calibration curve

Under ideal conditions increasing volumes of 100 μg .ml^{-1} of the IRB were added followed by

addition of 1.5 ml of 1 M HCl to Series of 25 ml volumetric flasks covered the range 1 - 35 µg.ml^{-1}, followed by adding 2.0 ml of 100 µg.ml^{-1} NBS , then was added. The solutions were kept at room temperature for 5 minutes, Then 2.5 ml of 100 µg. ml^{-1} RD then was waited for 5 minutes   before completed the volume to the mark by distilled

water. The absorbance was estimated against reagent blank at 577 nm. The producted calibration curve (fig. 6) shows that there is a linear relationship in the concentration range (1.0 - 25) µg. ml^{-1} with molar absorptivity 4.499×10^{4} L.mol^{-1}.cm^{-1} and sandell's sensitivity index 0.0095 µg.cm^{-2}.
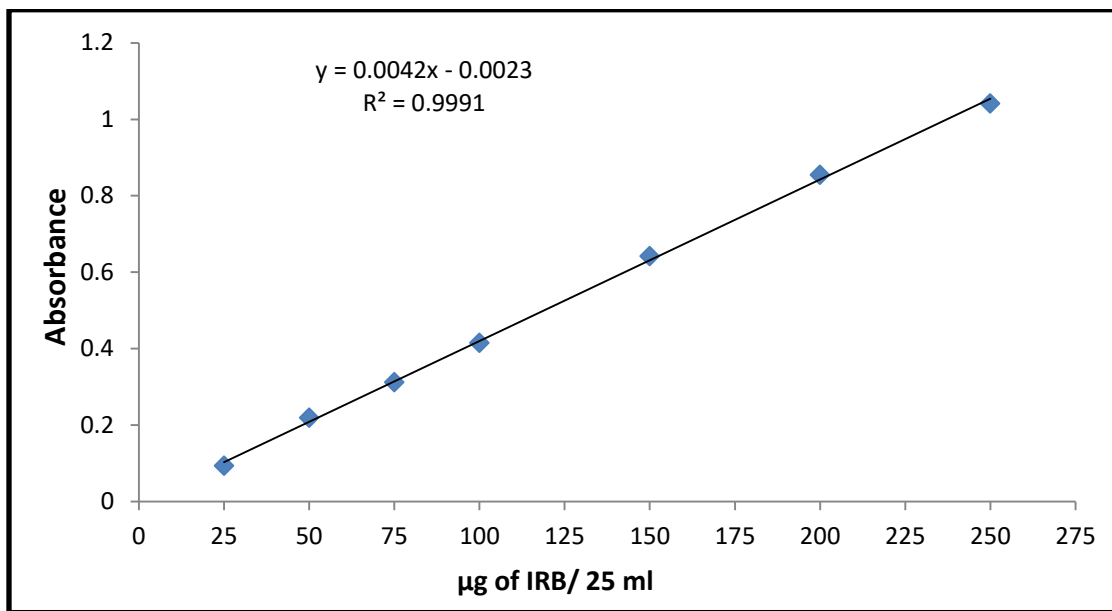

Fig. 6 :Calibration curve of IRB Estimation

Accuracy and precision:

To find out the accuracy and precision of the proposed method, two concentrations of IRB were determined. The results shown in (Table 6) show that the proposed method is reliable.

Table 6 :accuracy and precision

Amount of IRB µg/25 ml present	Amount of IRB µg/25 ml found	Recovery %	Rrelative error, %*	relative standard deviation, %*
100	99.07	99.07	-0.93	±1.659
200	192.89	96.45	-3.55	±2.446

 *Average of five determinations

Application the method :

The proposed method has been successfully applied to determine the drug in its pharmaceutical preparations (tablets). The results in (Table 7) show that the method has good accuracy and repeatability.

Table 7. Determination of IRB in pharmaceutical preparations

Drug	IRB Present (µg)	IRB Found (µg)	Relative error (%)*	Recovery (%)*	RSD*
GIZLAN (Tablets) 300 mg IRBN / 1 tab (JORDAN)	50	49.40	- 1.2	98.80	1.87
	70	69.76	- 0.34	99.66	1.62
	100	98.26	- 1.74	98.26	2.05
	200	194.93	- 2.54	97.46	2.99

*Average of four determinations

Evaluation of the proposed procedure

To ensure the selectivity of this method, the standard addition method was applied by adding a certain volume of the medicinal solution containing an appropriate amount of the drug to several 25 ml volumetric vials, then adding increasing amounts of the pure IRB solution and adding the other components of the procedure. Complete each vial to mark with distilled water and mix well. Complete each vial to label with distilled water and mix well, and the absorbance of the solutions was estimated as 577 nm. (Figure 7) and (Table 8) show the results of this study:
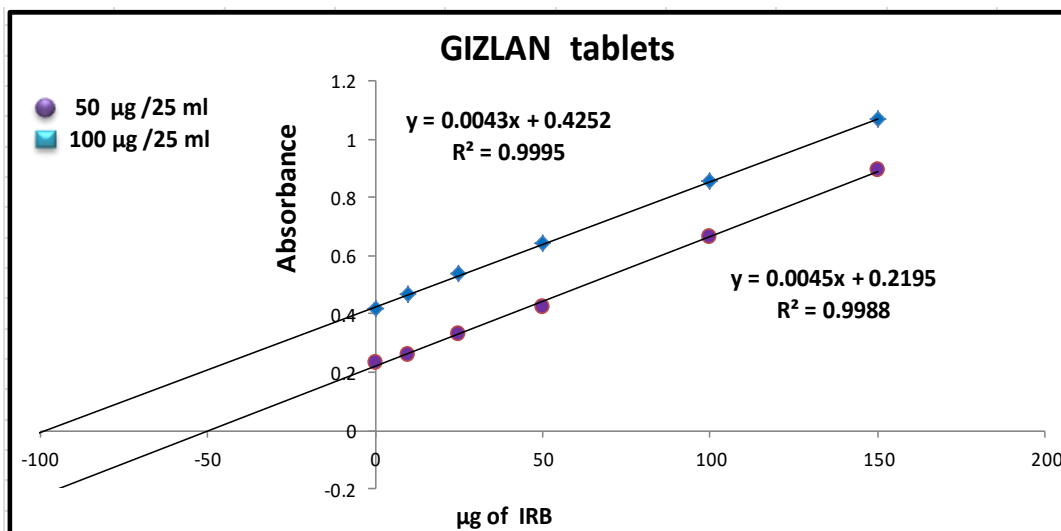

Fig. 7 : Calibration graphs of standard addition method for assay of IRB in GIZLAN tablets

Table 8. The results of standard addition methods for analysis of IRBN

Drug	IRBN Present (µg)	IRBN Measured (µg)	Recovery (%)
GIZLAN (Tablets) 300 mg IRBN / tablet. (JORDAN)	50	48.78	97.56
	100	98.88	98.88

The results included in (Fig. 7) and (Table 8) confirm that the proposed method has good selectivity.

4. Conclusion

A simple and sensitive spectrophotometric method was proposed for the indirect determination of Irbesartan based on its oxidation using known excess of N-bromoboxinamide and then determination of the residual N-bromoboxinamide by rhodamine dye, the method was sensitive, with acceptable accuracy and applicable to pharmaceutical preparations.

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